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Basic Concepts of Plant Disease and How to Collect a Sample for Disease Diagnosis[1]

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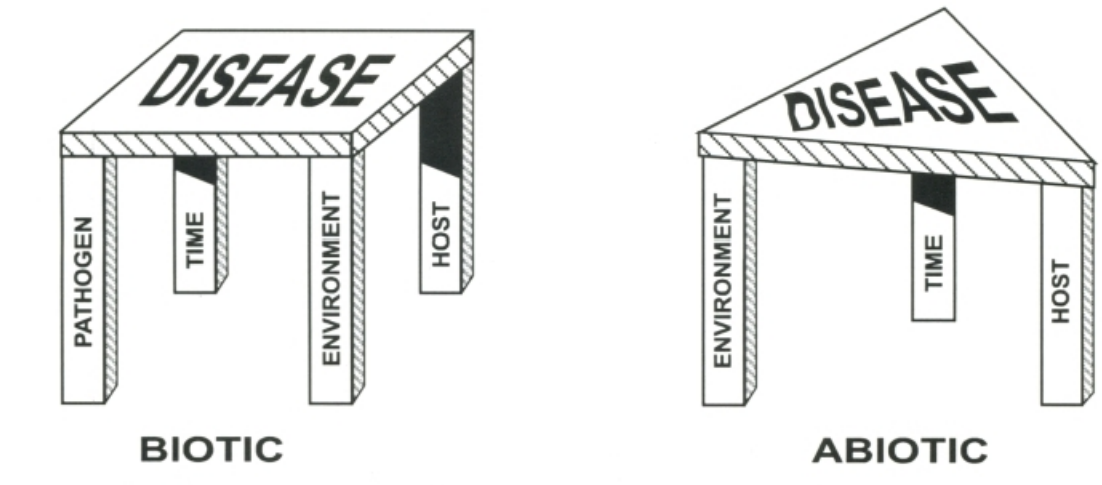
INTRODUCTION: This circular represents a departure from the usual format in which a particular plant disease is treated. The problem of inadequate sampling has always been one of the major obstacles in determining an accurate disease diagnosis. Ideally the diagnostician should collect the sample, but this is not always practical. Training of personnel responsible for sample collection to improve the quality of submissions would be incomplete without a concise discussion of basic concepts of plant disease, in order to rationalize the sample collection guidelines and procedures.

WHAT IS A PLANT DISEASE?

A modified definition of disease from Webster's Third New International Dictionary is: "Disease is an impairment of the normal state of the living (plant) that interrupts, modifies, (or stresses) vital functions. Disease is a response to specific causal agents (biotic or abotic), inherent defects of the organism, or combinations of these factors".

Diseases are recognized by the resulting symptoms expressed by the host, and signs (actual manifestations) of the causal agent. Taken together, symptoms expressed by the host and signs of the causal agent are called the disease **syndrome.** The causal agents, as the term is used in the above definition of disease, can be biotic (living or infectious), or abiotic (non-living or non-infectious). Biotic causal agents of disease are termed pathogens. Examples of biotic causal agents (pathogens) of plant disease are fungi, bacteria, mycoplasmas, spiroplasmas, viruses, viroids, nematodes, protozoans, and parasitic higher plants. Examples of abiotic causal agents of plant disease are temperature extremes, moisture extremes, nutrient extremes, light extremes, soil pH extremes, air pollution, oxygen deficiency, pesticide phytotoxicity, cultural malpractices.

Fig. 1. The biotic and abiotic disease tables.



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A visual aid to understanding all of the factors involved in the manifestation of both biotic and abiotic plant diseases are the plant disease tables shown in Figure 1. Each leg of the four-legged table represents one of the essential components for biotic disease development. Where the three components of virulent pathogen, susceptible host and conducive environment coincide for a sufficient period of time, disease will occur. In the simplest interpretation, lacking any one of the components will preclude disease development.

Abiotic disease development (disease caused by a non-living agent) can be similarly represented by a three-legged table. Where the two components of a susceptible plant (no longer a host in the strict sense) and harmful environment coincide for a sufficient period of time, such conditions will support the development of abiotic disease.

Inherent plant defects or genetic disorders which result in disease symptoms may be expressed under stable, innocuous environmental conditions, or may require some normally harmless abiotic or biotic stimulus for symptom expression. A few cases of disease induced by exopathogens (pathogen not in intimate contact with a susceptible plant) are known, and may have been mistakenly attributed to inherent plant defects or abiotic agents in the past. Additionally, there are examples of seemingly symptomless plant diseases that are not uncovered until yield losses are compiled, or the true healthy appearance of the plant is revealed (Fox, 1993).

Various symptoms of plant diseases are illustrated diagramatically in Table 1 together with possible causal agents and certain considerations to be weighed during the sample collection process. It is important to remember that even though a disease may appear to be static, as a rule diseases are dynamic, and the disease syndrome will change over time. Symptoms are usually not reversible, though with some abiotic causal agents such as drought stress or nutrient deficiency, symptoms (wilting, chlorosis) can be reversible. Of course, healing of the damage done by biotic and abiotic causal agents, or even by inherent defects in the host plant can occur, though some evidence of the disease usually remains.

While considerable literature has been generated on disease diagnostic techniques useful once the sample has been received in the clinic, (Burchill 1981; Dhingra and Sinclair 1985; Fox 1993; Johnston and Booth 1983; McIntyre and Sands 1977; Shurtleff and Averre III 1997; Tuite 1969; Waller, et al. 1998) , surprisingly little attention is given to the important initial step of the diagnostic process, the collection of the sample. **Without an intelligent approach to the acquisition of the sample, the accuracy of the entire diagnosis is suspect.**

Whereas it is helpful to know the mechanisms by which causal agents and inherent plant defects cause the symptoms of a disease, this information is not essential in order to obtain a good sample for diagnosis. Good observational and detective skills are necessary. The inspector/collector should have a good concept of the normal appearance of the plants under scrutiny, so that samples of deviations from normal can be collected. Some disease is to be expected in any plant population even if (sometimes especially if!) there is human interference. If symptoms are seemingly undetectable, the disease is either not serious enough to warrant attention, or observational skills need improvement. Some suggestions to improve observational skills are given in Table 4.

One common omission in inspection is examination of underground or ground level plant parts. Avoid the temptation to collect only symptomatic tissues, as these do not necessarily harbor the pathogen. Sampling techniques are unavoidably destructive to the host plant, sometimes quite so, using even the most sophisticated diagnostic methodology. If there is ever any doubt about what normal plant appearance should be, or what to include in a sample for diagnosis, **send** whole plants including roots **and soil** (about a liter of soil and roots), or **representative samples of all plant** parts and **soil** from plants that are too valuable, too large, or otherwise impractical to send to the clinic. (Other considerations for collecting samples are given in Table 1.)

Some diseases will inevitably defy complete diagnosis, therefore resampling may be necessary. Even then, no thorough resolution of the problem may be forthcoming. The diagnostic team should come closer to the answer(s) with each attempt, however. Diseases of complex etiology (more than one causal agent) often fall into this refractory category, and are actually quite common (Wallace 1978).

TABLE 1. SYMPTOMS, POSSIBLE CAUSES, AND SAMPLING CONSIDERATIONS

Symptom	Possible Causes, Sampling Considerations
 leaf spot	Fungal, bacterial, or viral leaf spot pathogen, chemical phytotoxicity. Collect all stages of development. Note pesticide schedule.
 leaf tipburn or scorch	Root or stem dysfunction, water stress, excess soluble salts, herbicide or other chemical injury. Fungal, bacterial or other pathogenic infection. Collect whole plant with roots and soil. Note pesticide and fertilizer schedule plus other components of cultural program.
 distortion	Fungal, bacterial, viral or other pathogenic infection, herbicide or growth regulator injury, insect or mite damage, chemical phytotoxicity, mechanical damage. Collect representative sample of all symptoms. Note cultural program.
 vein enation	Viral infection, growth regulator injury. Collect representative range of symptoms. If possible, keep tissue fresh or submit whole plant to permit observation and testing to take place over a period of time.
 edema	Excess moisture, chemical phytotoxicity, fungal infection. Collect all representative stages. Note soil moisture, preceding weather conditions (prolonged cloudy, humid to wet weather?).
 vein clearing	Viral infection, growth regulator or herbicide injury. Collect all representative stages, note pesticide schedule on or near crop. Keep sample very fresh (essential for many viral diagnostic tests). Whole plant helpful.
 mosaic ringspot	Viral infection, reaction to cold or hot water on foliage, chemical phytotoxicity. Note watering methods, pesticide schedule. Collect very fresh sample or whole plants.
 mildew	Fungal infection. Collect symptomatic tissues.

leaf blister

Fungal infection, insect or mite injury. Collect range of symptomatic tissues.

scab

Fungal or bacterial infection, insect or other injury, chemical damage. Collect range of symptomatic tissues.

defoliation

Root dysfunction, water stress, excess soluble salts, cold, heat, normal senescense, chemical or insect damage. Collect whole plants. Note recent cultural treatments.

chlorosis

Macro- or micronutrient deficiency, low or excess light, growth regulator or herbicide injury, natural pigmentation/variegation, root dysfunction, soil pH problems. Collect representative samples of all symptoms, soil and roots.

wilt

Fungal, bacterial, viral or other pathogenic wilt, root rot or canker pathogen. Water stress, excess soluble salts, high temperature, wind, cold, insect injury. Implies sudden onset. Collect whole plant with roots and soil.

tip dieback

Slower or later expression of same factors as above, plus cold injury, insect damage.

blight

Localized or generalized branch or twig disorder caused by fungi, bacteria, insects, mechanical injury. Collect sample which includes transition zone from diseased to healthy tissue.

stunt

Fungal, bacterial, viral or other pathogenic infection, nutrient deficiency, water stress, nematode or insect injury, growth regulator damage. Collect whole symptomatic and asymptomatic plant. Note cultural program.

fasciation

Unknown. Usually considered a genetic abnormality, sometimes associated with insect injury. Collect symptomatic tissue of all stages, whole plants if possible. Note pesticide and growth regulator schedule.

proliferation witches' broom

Fungal, bacterial, viral, or MLO infection. Mite infestation. Micronutrient deficiency, herbicide or growth regulator phytotoxicity. Collect whole plants if possible. Note pesticide schedule.

girdle

Fungal disease, mechanical constriction, ground-line heat canker, insect injury. Collect symptomatic tissues of all stages of development.

canker diffuse and/or calloused

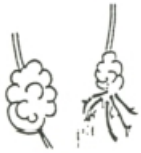
Fungal or bacterial infection, mechanical injury, heat or cold injury, sunburn, chemical injury. Collect symptomatic tissues, especially transition zones between healthy and symptomatic tissue. Check pattern of symptom expression.

Initiation of root primordia

Excess water, mechanical or chemical injury or pathogenic infection at ground line. Enough vigor to induce root primordia implies sudden onset of disorder to otherwise healthy plant. Collect whole plant or all representative plants parts.

pustule

Fungal infection (rust), excess moisture (exploded lenticels). Collect symptomatic tissue.



gall

Fungal or bacterial infection, mechanical injury, hormonal disruption, insect injury. Collect samples of all stages of gall development, including surrounding soil if below ground.

root rot

Fungal or bacterial infection, excess moisture, excess soluble salts, chemical injury. Collect whole plant with soil and roots.

nodule root galls

Nodulating N-fixing bacteria, actinomycetes, bluegreen algae, root-knot nematode, insect injury, fungal bacterial or viral pathogenic infection. Collect symptomatic tissue.

crown rot bud rot

Crown rot affects oldest tissues. Bud rot affects youngest tissue. Fungal, bacterial infection. Excess soluble salts. Collect entire plant with roots and soil.

dry rot

Fungal infection, mechanical injury, heat or cold damage, chemical injury. Collect representative samples of all stages.

smut

Fungal infection. Collect representative samples of all stages.

PROCEDURE FOR SAMPLE COLLECTION, PACKAGING AND SUBMISSION

A suggested list of supplies to include in a field kit used for collecting diseased plant samples is given in Table 2. After deciding what to include in the sample, the following procedures for obtaining, packaging, and submitting the sample are suggested:

1) Obtain fresh material in reasonable quantity, several examples of the various symptoms being expressed. Be certain to include as many identifiable stages of the disease as are represented. Most recently developed symptoms usually afford the best material for diagnosis. Be sure to include suitable plant material for botanical identification, since occasionally field identifications may be in error or the host plant identity may not be known.

2) Lift roots carefully so as not to leave feeder roots or rotted roots behind. Include about a liter of soil for pH, soluble salts, and possibly a nematode assay.

3) Place samples in appropriately sized plastic bags, including a paper towel for a blotter if sample is very wet. Duplicate dry samples are recommended if the sample is succulent or fragile.

4) Wrap a wire twist-tie around stem at ground line to keep soil off of above-ground plant parts. Accurately label samples. Place the entire sample in a paper bag or an unsealed plastic bag.

5) Keep samples cool, protected from crushing.

6) Gather appropriate information from the grower (see Table 3) and make pertinent observations (see Table 4). Include this information with sample submission form. Complete mailing addresses and map locations are necessary if owners want to be informed of diagnosis or site must be revisited. Also, clerical work in the clinic is greatly simplified when a properly completed submission form is included with the sample.

7) Deliver promptly to the clinic. Next day service is recommended.

8) TABLE 2. CONTENTS OF A FIELD KIT FOR PLANT DISEASE SAMPLE COLLECTION

Plastic and paper bags	Pruning shears, pruning saw	Wire twist ties
Paper towels	Knife, hatchet, increment borer	Plastic/rubber gloves
Trowel, shovel, soil probe	Rubber bands	Hand lens 5-10X
Forms, pencil	Labels	Vials/jars with lids
Padded collection boxes	*Camera, film, macro lens flash equipment	Large ice chest/cooler
Maps	Field manuals, compendia	Disinfectant for hands & tools
*Light meter	*Portable soil pH and soluble salts meter	

*Optional

TABLE 3. EXAMPLES OF INFORMATION INSPECTOR SHOULD OBTAIN FROM GROWER

1. Host: cultivar/variety; age; propagation method; site prep; names; dates and rates of fertilizer, pesticide history and schedule*, growth regulator applications; pruning; pinching; transplant; omissions or additions to usual/conventional culture program.

2. Irrigation: frequency, rate, timing; water quality; determination of need.

3. Soil/media: pH, soluble salts, texture, drainage, homogeneity, aeration, temperature, planting depth, cultivation, cropping history, earthmoving/construction, burial/disposal site, trash burning.

4. Environmental conditions: temperature, humidity, ventilation, wind, lightning, exposure, light intensity, air quality, method of heating and cooling, etc., all preceding and during syndrome development.

5. Date of first symptoms and rate of syndrome development, coincident with any treatment or environmental event.

6. Recent human, animal, insect, mite activity around or on symptomatic plants.

7. Idiosyncracies of plants in question, predisposition (plant made more susceptible) by cultural or environmental conditions.

"Use appropriate safety precautions when handling pesticide treated crops.

TABLE 4. EXAMPLES OF OBSERVATIONS TO BE MADE BY INSPECTOR

1. Spatial and chronological pattern of disease in crop, on individual plants. Are symptoms different or consistent, uniform or scattered? Are symptoms spreading across crop or progressing on individual plants?

2. What is the frequency and intensity of the syndrome?

3. Any signs of pathogen/causal agent? (fruiting structures, chemical residues, insects/mites, frass, etc.).

4. Any evidence of host recovery?

5. Nearby plants (same or different) show any symptoms? Are root zones shared?

6. Inspect interior, crown, roots of plant. Cut open stems, crowns, flowers, fruits, and roots. Are there any hidden, internal symptoms/signs of disease?

CONCLUSIONS: The whole process of sample collection for disease diagnosis is of pivotal importance. Unless done correctly, the entire diagnostic process may be side-tracked, and the subsequent recommendations for controlling or correcting the disease may be of limited effectiveness. Sample collection can be a difficult, time-consuming process, and there is no guarantee that the plant problem in question will be resolved with a single sampling. With all the variables involved in plant production, every plant problem demands different sampling techniques. By justifying and summarizing the various phases and techniques of the plant disease sample collection process, inspectors should enjoy greater efficiency, and more accurate diagnosis and resolution of the plant disease problems they encounter in the field.

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